INFLUENCE OF THE SINUSGLAND OF CRUSTACEANS ON NORMAL VIABILITY AND ECDYSIS[1]

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Since the work of Perkins and of Koller in 1928, who independently described the presence of a substance in eyestalk extract which exercises a very potent effect upon the chromatophores of crustaceans, there has been much interest shown in the crustacean eyestalk function. The picture has been rendered even more interesting as a result of the work of Brown (1935) and of Kleinholz (1938), demonstrating that humoral activity in this group of animals is by no means a simple one, but that several hormonal substances are normally functioning. Hanström (1935) performed experiments in which he showed that the portion of the eyestalk which was active in affecting chromatophores always contained, among other things, a tissue which he has termed the sinusgland. This has given rather good evidence indicating which tissue of the eyestalk is the active one in this regard. The cells of this tissue were shown to be secretory in nature and to contain a rich supply of secretory granules. The more recent work of Hanström (1936), Stahl (1938) and others have shown the sinusgland to be present in some degree in all the crustaceans that have been examined in detail. Its occurrence appears to be quite independent of the state of development of a chromatophore system. Functionally it appears to have common properties with the corpora allata of insects since an extract of the latter organ in many cases serves as an activator of crustacean chromatophores. Abramowitz (1936, 1938) has demonstrated that the chromatophorotropic substance from the sinusgland and the intermedin of the vertebrates have certain common chemical and physiological properties.

Koller (1930) was the first to demonstrate that the eyestalk substance has another function in addition to the control of chromatophores. He found that animals from which the eyestalks had been removed failed to deposit calcium in their exoskeletons to the same extent as normal animals. He interpreted this to be the result of removal of the source of a controlling hormone. Welsh (1937) found that when he perfused an exposed crayfish heart with eyestalk extract

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there was a pronounced speeding up of that organ. Brown (1938) demonstrated that removal of the eyestalks appreciably shortened the life of the individual and that the shortening thus induced could be compensated for in part by implantation of eyestalk tissue into the ventral abdomen. This shortening of the life of the animal has been called a "viability effect" of an eyestalk hormone, though it is fully realized that this is a function described in far too general terms. It is hoped that this "viability effect" can soon be analyzed into the particular phenomena responsible for the shorter life.

There has frequently been suggestion of a "molting effect" of the eyestalk substances, though no adequate data have yet been published to establish such a function. The only grounds for such a belief are that several investigators have mentioned that eyestalkless animals appear to molt more frequently than normal ones. No reason has been advanced for thinking the effect is due to anything other than the injury caused by the operation of eyestalk removal (indicated by Darby, 1938).

The following research has been conducted in continuation of that of Brown (1938) with the intention of discovering just what tissue of the eyestalk is responsible for the "viability effect" of this organ. There is included here the first direct evidence for an endocrine activity of the sinusgland of the crustacean. Hitherto its functioning had been supposed upon the grounds of the best of circumstantial evidence. During these experiments the sinusgland has been dissected out and implanted into the ventral abdominal sinus of eyestalkless animals. Direct physiological evidence of its endocrine function has been demonstrated. Furthermore, it is quite well established as a result of these experiments that this gland is the one responsible for the normal continuation of life of the animal and also that it has a functional activity in the control of molting. The possibility of explaining the viability effect of eyestalk hormones in terms of molt control will be discussed.

Methods and Materials

All the crayfishes used in these experiments were small individuals (carapace lengths 15–30 mm.) of the species *Cambarus immunis*, with the exception of certain large individuals (*Cambarus virilis*, *C. blandingii*, and *C. immunis* of carapace lengths 30–40 mm.) which were used as the source of the sinusgland for implantation. The animals were brought into the laboratory a few days before the beginning of an experiment. It was our purpose to use experimental extirpation and implantation to determine the normal functions of the eyestalk gland within the body.

The method of extirpation was simple: the eyestalks were removed as a whole and the wound sealed with an electric cautery. By so sealing the wound, less than 10 per cent of the animals died as a result of the operation. It is fully realized that such a method of gland extirpation removed much tissue in addition to that of the sinusgland.

In the first experiment to be described the implantation consisted of all the eyestalk tissue. The eyestalks were removed from an animal and dropped into amphibian Ringer's solution. With the aid of a dissecting microscope the exoskeleton of the eye was cut away. The soft parts of the eyestalk were easily removed with fine forceps. This tissue was then teased into minute fragments and injected by means of a glass capillary pipette into the ventral sinus of the abdomen. The glass pipette proved to be especially satisfactory since it was possible to ascertain that all of the tissue entered the animal and none was left adhering to the walls of the pipette.

In those experiments in which the sinusgland by itself was to be implanted the gland was carefully dissected out in the following manner: the eyestalk was removed from a large crayfish and dropped into a watchglass containing amphibian Ringer's solution or a balanced salt solution based on Griffeths' analysis of *Astacus* blood (which will henceforth be referred to as Griffeths' solution). With a pair of sharp pointed scissors the chitinous exoskeleton was clipped to free the dorsal half of the stalk skeleton from the ventral half. The contents of the stalk were then picked out with fine pointed watch-maker's forceps and the dorsal tissue was teased away in the direct light of a strong lamp. The sinusgland tissue stood out quite conspicuously as a seemingly fibrous and granular bluish tissue. This mass of tissue was easily torn away from the adjacent nerve tissue. All the adhering tissue was teased away and the gland rinsed in amphibian Ringer's or Griffeths' solution. With forceps the gland was next pushed through an opening made in the ventral side of the abdomen. The clear exoskeleton in this region made it possible to ascertain that the minute gland was actually left in place upon removal of the forceps.

In order to determine the exact location of the tissue removed from the eyestalk, sections were made of the bluish gland-like tissue that was removed, and also of all the remaining portions of the eyestalk. In addition, longitudinal sagittal sections of the complete eyestalk were made as a control. By study of these three sets of sections it was readily determined just what tissue was being implanted. It was discovered that the implant tissue in histological section appeared to be definitely glandular in nature and occupied a position wedged between the medulla externa and the medulla interna. Con-

sidering its position and the fact that its cytoplasm was richly charged with eosinophilic inclusions, it seemed highly probable that this gland was the same as that described by Hanström (1936) as the sinusgland. The accompanying photographs show this gland as it occurs in *Cambarus virilis*. The first photograph is a median sagittal section of the

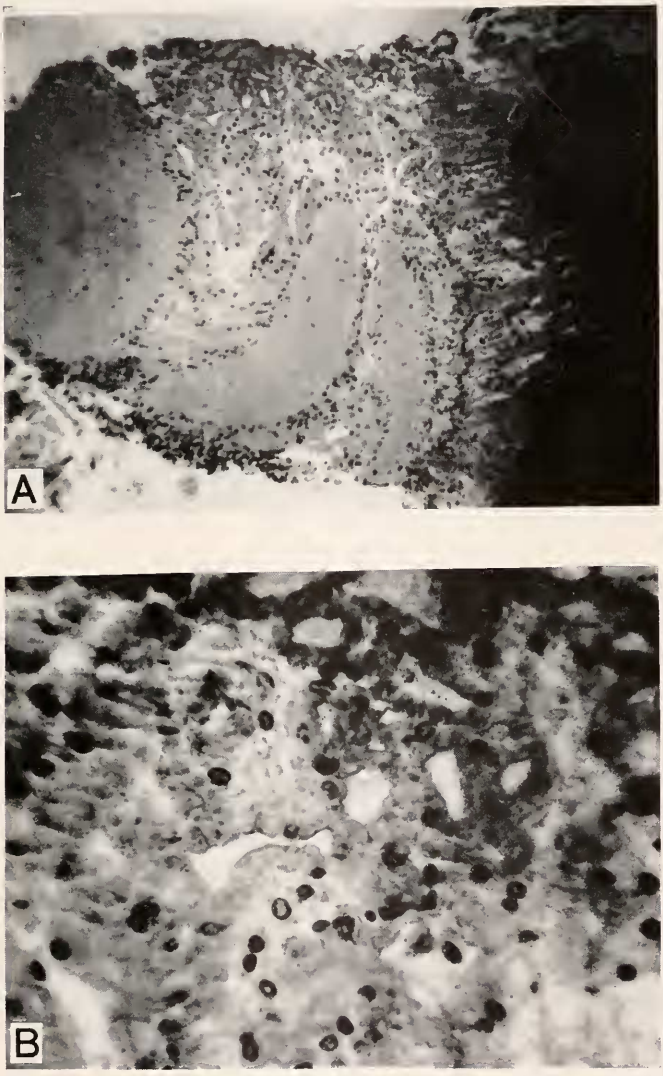

FIG. 1. Sagittal sections through the eyestalk of *Cambarus virilis* (6 micra thick and stained with Delafield's haematoxylin and eosin). *A*. At a magnification of 80 ×, showing the sinusgland as a somewhat triangular section of tissue located dorsally to a point intermediate between the medulla externa and the medulla interna. *B*. A higher magnification (360 ×) of the central region of the sinusgland.

eyestalk at a magnification of approximately 80× and the second is a higher power magnification (about 360×) in the central region of the gland.

During the experimental period all the animals were kept in individual glass finger bowls in water not quite deep enough to cover the carapace. These finger bowls were covered loosely with glass plates to minimize evaporation of the water but still to permit circulation of air over the water surface.

The experiments performed included extirpation and implantation, with appropriate controls, and observations were made upon viability and molt behavior.

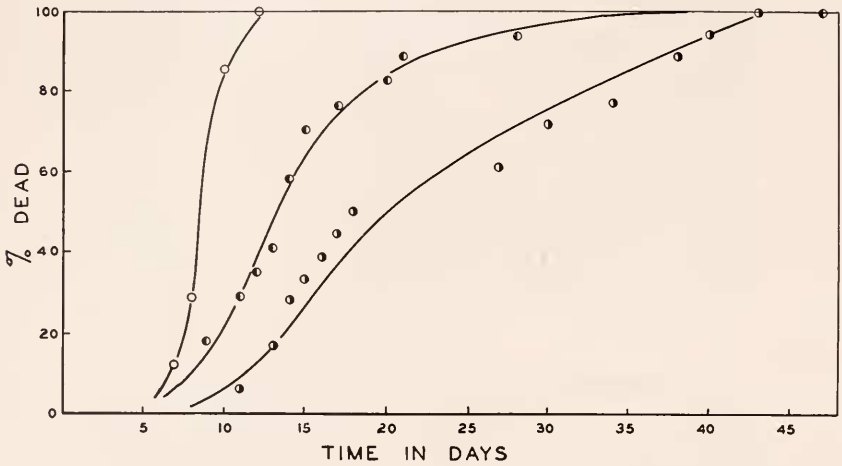

FIG. 2. The relation between the percentage of animals dead and the number of post-operative days for eyestalkless crayfishes, (○); eyestalkless crayfishes with a heteroplastic implant of sinusglandless eyestalk tissue, (◖); and eyestalkless crayfishes with only a heteroplastically implanted sinusgland, (◗).

EXPERIMENTS ON VIABILITY EFFECTS

Experiment I

The animals of this experiment, all *Cambarus immunis* with both eyestalks removed and the stubs cauterized, were divided into three lots. In the first lot were 7 animals with no further treatment. The second lot of 17 animals had a sinusgland taken from a single eyestalk of a large *Cambarus virilis* or *Cambarus blandingii acutus* implanted into the ventral sinus of their abdomens. The third lot of 18 animals had an abdominal implantation consisting of all the eyestalk tissue of a single eyestalk of *Cambarus virilis* or *Cambarus blandingii acutus*, from which the gland had been carefully removed.

The results of this experiment are best shown in the form of a graph (Fig. 2) in which the percentage of animals dead is plotted

against the post-operational day. This graph demonstrates clearly that eyestalkless animals without abdominal implants live significantly shorter lengths of time than eyestalkless animals into which eyestalk tissue *minus the sinusgland* has been implanted. Similarly, eyestalkless animals which have received abdominal implants of the minute sinusgland by itself, live very significantly longer than those animals into which the remaining portion of the eyestalk tissue was implanted. Comparing only the instance of sinusgland implant with the case of no implant, we can conclude definitely that the minute sinusgland lengthens the post-operative life of the animal considerably. It is well to bear in mind that these two latter groups have been subjected to operations of different degrees of severity, in which the animals which

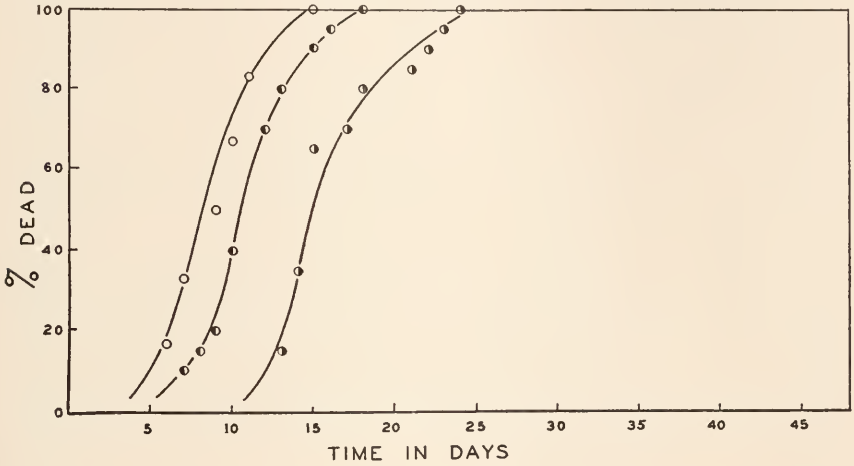

FIG. 3. The relation between the percentage of animals dead and the number of post-operative days for eyestalkless crayfishes, (○); eyestalkless crayfishes with a homoplastic implant of sinusglandless eyestalk tissue, (◐); and eyestalkless crayfishes with only a homoplastically implanted sinusgland, (◑).

live longer have been subjected to more severe operative injury, the animals of the latter group having their abdomens punctured as well as having both eyes removed. A logical explanation of the intermediate length of post-operative life in the instance of those animals with the glandless stalk tissue implants is that there is present in the blood spaces of the general eyestalk tissue a product that has arisen from the sinusgland. During its removal, a bluish liquid is seen to diffuse out of the gland and infiltrate into the surrounding tissues. The general stalk tissue is frequently filled with a homogeneous blue liquid which, in all probability, comes from the same origin. We believe, therefore, that this additional substance is responsible for permitting these animals to live longer than those in which no implant

is made. It is also possible that fragments of the gland itself still remain which were not removed at the time of operation.

The implants in this experiment are heteroplastic, while in an experiment to be described later all the implantations were autoplastic as was the case with those observations published by Brown (1938). It becomes doubly interesting that the sinusgland has a definite effect not only upon the length of post-operative life in the same species of animal, but that the tissue from one species is capable of working effectively within the body of another species to the same end. Thus these substances, or this substance, is inter-specifically active.

Experiment II

In this experiment, like the preceding one, eyestalkless *Cambarus immunis* were divided into three lots. In the first lot, consisting of 6 large animals, there was no further treatment. A single sinusgland from an eyestalk of a large animal of the same species was abdominally implanted into each of the 20 small animals of the second lot. Each of the 20 animals of the third group received an abdominal implant consisting of the tissue from a single large eyestalk from which the gland had been removed.

The results of this experiment are shown in Fig. 2.

This experiment confirms the influence of the sinusgland on viability demonstrated in Experiment I. Here the implantations were homoplastic, from large *Cambarus immunis* to small *Cambarus immunis*. As in Experiment I, the animals without any implant lived a much shorter time than those with sinusgland implants, and animals in which sinusglandless eyestalk tissue was implanted lived for an intermediate length of time.

Experiments on the Molting Control Function of the Sinusgland

Experiment I

This experiment was intended to discover any differences that might occur in the molting process among animals from which both sinusglands had been removed, one sinusgland removed, both sinusglands removed but with them autoplastically implanted into the ventral abdominal sinus, and finally, completely normal animals.

In this experiment four lots of animals were isolated. The first lot of 34 animals was left in perfectly normal condition, though placed in the usual individual glass finger bowls with covers. The second lot of 48 animals was subjected to removal of one eye each. A third lot of 79 animals had both eyestalks removed in the usual manner. The

fourth lot of 44 animals had both eyestalks removed and the contents of their own eyestalks in amphibian Ringer's solution injected into the ventral sinus of the abdomen. Observations were made only with regard to actual molting. The results that were obtained are summarized in Table I.

TABLE I

Data indicating the extent of molting in crayfishes under different experimental conditions.

	Normal Animals	One Eye Off	Two Eyes Off	Two Eyes Off (Implant)
Total no. examined....	34	48	79	44
No. "molts".....	9	19	23	3
Per cent "molts".....	26	40	29	7
Per cent "molts" dying in process.....	44	16	74	100
Per cent molt/av. life span.....	2.0	3.4	5.75	1.0

All the records of molting in Table I indicate instances in which the animal either completed the molt or was well along in the process at the time of death. The most significant portion of the table is the item "per cent molt/average life span" which gives the only true figure of the relative rates of molt. The "per cent molts" fail to do this inasmuch as the different lots of animals survived different lengths of time; consequently such animals as normal animals and those with one eyestalk off had a longer time in which molts could occur. On this strictly relative behavior (per cent molt/average life span) the figure for normal animals is 2. With one eye removed, the rate of molt is increased by about 75 per cent, and with the removal of two eyes the molting has been accelerated about 200 per cent. The striking fact, however, is that when both eyes were removed and the eyestalk tissue abdominally implanted, the figure indicating the molting rate is 1, or about half that of normal animals. Were it not for the anomalous molting rate of this last group the results could be interpreted as indicating that the rate of molting is a function of the extent of injury. But, taking the data together, there appears to be a more probable explanation. The eyestalk tissue, under nerve control, liberates a humoral substance into the blood which inhibits the molt. With one eye removed, relatively less substance is liberated and with two eyes removed none of the material, and we see molt correspondingly going on at relatively greater rates. In these terms the explanation of behavior of the last group of animals might be that the implanted glandular tissue continuously liberates some antimolting substance and the animal is almost unable to molt.

Some of the acceleration resulting after eyestalk removal may be due to injury effects, but that they are not totally due to injury is indicated by the implantation experiments.

Experiment II

This experiment points to the sinusgland in the eyestalk as the actual tissue involved in the formation of the molt control humoral substance. The data for this conclusion are taken from observations on molting in the animals in Experiment II on viability.

A consideration of the ratio of percentage of completed or nearly completed molts to average survival period, shows that the implantation of the sinusgland reduces the molting rate to about one-fifth of that which occurs in the controls with the glandless stalk tissue implants. The conclusions of the former experiment are confirmed and it is further indicated that the sinusgland is the effective tissue in molt control. The results of this experiment are summarized in Table II.

TABLE II

Data indicating the extent of molting in crayfishes under different experimental conditions.

	Two Eyes Off	Two Eyes Off (Implant)
Total no. examined	20	20
No. "molts"	4	1
Per cent molts	20	5
Per cent molts/av. life span	1.57	.31

In the course of this experiment all the animals were carefully watched, not only for completed molts but also for the slightest symptoms of the beginnings of molt. The early signs of molt were usually indicated by a visible separation between the carapace and the first abdominal tergite. Practically all of the eyestalkless animals, regardless of the type of implant, showed this separation from three hours to three or four days prior to their death. This was so definite that it was possible to predict the death of any animal within these limits. In many instances this separation was followed by a completed molt, though in the majority of cases the animals died before further steps in the molting process. It is admitted that some other factors, such as change in general tone of the abdominal musculature or upset in the water metabolism of the animal, might be operating in inducing the separation of these two skeletal elements. Superficially, however, we are unable to differentiate between the initiation of the normal molt and its induction by other causes. Furthermore, many of the animals showing this apparent initiation in the molt process showed

muscular activity of the body such as is usually associated with the normal molting process.

Those animals from which the eyestalks had been removed and which received the glandless eyestalk implantation, all showed the apparent initiation of molt or completed the molt prior to their death. In three cases the animals completed the molt before death, in one case dying within a day of the molt and in the other cases living two and four days, respectively, after molting. In a fourth case the animal died when well along in the molting process. These facts would indicate that even without the eyestalks the animals are physiologically able to complete the molt. But the fact that the eyestalkless animals sometimes continue to live several days after molting and then die without showing further signs of molt, indicates that the sinusgland has a function in addition to molt control.

The majority of the animals with sinusgland implants also showed the beginnings of molting prior to their death, just as did the first lot. The only difference between the lots seemed to be that the molting activity was postponed in the case of the implanted animals. These animals seldom do more than show this first sign of molt, scarcely ever proceeding far into the molt or completing it. A possible explanation of this is that these animals are prevented from molting by action of the implant until the absence of the eyestalk has worked other degenerating effects upon the organisms to the extent that they no longer have the power to go far with the molt, in spite of removal of the inhibitor through loss of function of the implant. In this regard it would be interesting to trace the rate of degeneration of the implanted tissue to see if there may be any correspondence between the time of oncome of the molt and the structural degeneration of the implanted cells.

It may be possible to interpret the data of Koller (1930) in terms of molt control activity. Animals molting more frequently as a result of absence of a hormone from the sinusgland might well be expected to have less calcium salts in their exoskeleton than normally.

Summary

1. Direct evidence for an endocrine activity of the crustacean sinusgland is given. This evidence has originated from implantation experiments.

2. Removal of the sinusgland significantly shortens the life of the animals, and conversely the length of life of animals with sinusglands removed can be significantly lengthened by implantation of the gland.

3. The sinusgland is readily dissected out in fresh eyestalk tissue

in strong reflected light. It has a distinctly bluish cast. It is a definite organ which can be readily teased away from the surrounding tissue and removed as a whole.

4. Certain evidence suggests very strongly that a substance concerned with the control of molting is elaborated in this gland. The most probable action of this substance is that of inhibiting molt.

5. The action of the sinusgland in molt control appears to be insufficient to explain the viability effect entirely.

LITERATURE CITED

Abramowitz, A. A., 1936*a*. Action of crustacean eye-stalk extract on melanophores of hypophysectomized fishes, amphibians, and reptiles. *Proc. Soc. Exp. Biol. and Med.*, pp. 714–716.

Abramowitz, A. A., 1936*b*. The action of intermedin on crustacean melanophores and of the crustacean hormone on elasmobranch melanophores. *Proc. Nat. Acad. Sci., Washington*, **22**: 521–523.

Abramowitz, A. A., 1938. The similarity between the hypophyseal chromatophorotropic hormone and the chromatophorotropic hormone of the crustacean eyestalk. *Physiol. Zoöl.*, **11**: 299–310.

Brown, Frank A., Jr., 1935. Control of pigment migration within the chromatophores of Palaemonetes. *Jour. Exper. Zoöl.*, **71**: 1–15.

Brown, Frank A., 1938. An internal secretion affecting viability in Crustacea. *Proc. Nat. Acad. Sci., Washington*, **24**: 551–555.

Darby, Hugh H., 1938. Moulting in the Crustacean, Crangon armillatus. *Anat. Rec.*, **72**: (Suppl.) 78.

Hanström, B., 1935. Preliminary report on the probable connection between the blood gland and the chromatophore activator in decapod crustaceans. *Proc. Nat. Acad. Sci., Washington*, **21**: 584–585.

Hanström, B., 1937. Die Sinusdrüse und der hormonal bedingte Farbwechsel der Crustaceen. *Kungl. Svenska Vetenskap. Handl.*, Ser. 3, **16** (3): 1–99.

Hanström, B., 1937. Vermischte Beobachtungen über die chromatophoraktivierenden Substanzen der Augenstiele der Crustaceen und des Kopfes der Insekten. *Kungl. Fys. Sällsk. Handl.*, **47** (8): 3–11.

Kleinholz, L. H., 1938. Studies in the pigmentary system of crustacea. IV. The unitary versus the multiple hormone hypothesis of control. *Biol. Bull.*, **75**: 510–532.

Koller, G., 1928. Versuche über die inkretorischen vorgänge beim Garneelenfarbwechsel. *Zeitschr. f. vergl. Physiol.*, **8**: 601–612.

Koller, G., 1930. Weitere Untersuchungen über Farbwechsel und Farbwechselhormone bei Crangon vulgaris. *Zeitschr. f. vergl. Physiol.*, **12**: 632–667.

Perkins, E. B., 1928. Color changes in crustaceans, especially in Palaemonetes. *Jour. Exper. Zoöl.*, **50**: 71–103.

Stahl, Filip, 1938*a*. Preliminary report on the colour changes and the incretory organs in the heads of some crustaceans. *Arkiv. för Zoologi*, 30B: 1–3.

Stahl, Filip, 1938*b*. Über das Vorkommen von inkretorischen Organen und Farbwechselhormonen im Kopf einiger Crustaceen. *Kungl. Fys. Sällsk. Handl.*, **49** (12): 3–20.

Welsh, J. H., 1937. The eyestalk hormone and rate of heart beat in crustaceans. *Proc. Nat. Acad. Sci., Washington*, **23**: 458–460.